



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

NUCLEAR BEHAVIOR IN THE PROMYCELIA OF CAEOMA NITENS BURRILL AND PUCCINIA PECKIANA HOWE¹

LOUIS OTTO KUNKEL

In a preliminary paper (10), I have shown that the aecidiospores of *Caeoma nitens* Burrill produce promyelia. The behavior of the nuclei during the germination of these spores was not described at that time and has been the subject of my further studies.

Tulasne (19) in 1854 found that the aecidiospores of *Endophyllum Euphorbiae silvaticae* do not germinate as normal aecidiospores. Instead of forming non-septate germ tubes, these spores produce promyelia. Morphologically considered they are aecidiospores; nevertheless, they function as teleutospores. Sappin-Trouffy (17) also studied them and confirmed the observations of Tulasne as to the manner of their germination. According to Sappin-Trouffy, however, the behavior of the nuclei in the germination of this aecidiospore differs from that in a normal teleutospore. The two nuclei in this case do not fuse but pass directly out into the tube of the promycelium where they divide to produce the four nuclei.

Maire (12) also made a cytological study of the aecidiospores of *E. Euphorbiae silvaticae*. He agrees with Sappin-Trouffy that nuclear fusions do not occur in them. He also studied the aecidiospores of *E. sempervivi* and decided that here again the nuclei do not fuse but pass out into the germ tube and divide, just as in the case of *E. Euphorbiae silvaticae*.

More recently Hoffmann (8) has made a very careful cytological study of *E. sempervivi*. He finds that a nuclear fusion does occur in the aecidiospores of this rust and that the four nuclei of the promycelium arise by two successive divisions of the fusion nucleus, just as in the case of ordinary teleutospores. He showed that the first or even both of these divisions may take place in the aecidiospore. This observation suggests that the two nuclei, which according to Maire (12) pass out of the spore into the promycelial tube are the product

¹ Contributions from the Department of Botany, Columbia University. No. 259.

of the first division of a fusion nucleus. In the light of Hoffmann's work it seems highly probable that nuclear fusions also occur in the aecidiospores of *E. Euphorbiae silvaticae*. Sappin-Trouffy (17) and Maire (12) were probably both wrong in supposing that the nuclear phenomena connected with the production of promycelia by the aecidiospores of *Endophyllum* are different from those to be observed in the case of teleutospores.

Although the production of promycelia by the aecidiospores of *E. Euphorbiae silvaticae* and *E. sempervivi* is well established, certain observations have been made which have been regarded as indicating that these aecidiospores may at times germinate in the ordinary fashion. Thus Nypels (15), Maire (12), and Werth (20) claim that occasionally they find non-septate germ tubes which are like the germ tubes produced by the aecidiospores of other rusts. Werth noted that the aecidiospores of *E. sempervivi* produce non-septate germ tubes when immersed in water during germination, but that if floated on the surface of the water, they produce typical promycelia. He seems inclined to think that these non-septate germ tubes can infect the host plant. Hoffmann (8), however, has failed to find any evidence that the aecidiospores of *E. sempervivi* ever produce the ordinary type of germ tubes. It is evident that a further cytological study of these aecidiospores is needed to settle this important question. If it could be shown that nuclear fusions occur in them and that their tubes typically contain four nuclei, which are the product of two successive divisions of this fusion nucleus, then, even in the absence of septa, the promycelial nature of the tubes would be established. If on the other hand it could be shown that nuclear fusions do not occur under the conditions mentioned, but that the two nuclei pass out into the germ tube and there divide conjugately, we should be compelled to admit that the type of germination is dependent on environmental conditions. In the absence of direct cytological evidence, however, we must be very slow to accept such claims.

My observation that the aecidiospores of *Caeoma nitens* Burrill also produce promycelia, indicates that this rust has a life history similar to that of the two species of *Endophyllum* noted above. *Caeoma nitens* is very widely distributed and produces aecidiospores in great abundance. It thus furnishes excellent material for the study of the questions that have been raised in connection with the genus *Endophyllum*. I have described the earlier work on *C. nitens* in my pre-

liminary paper (10). Galloway (14) studied the germination of the aecidiospores in water and noted that the germ tubes which they produce may become somewhat septate, but he did not observe the production of sporidia and his drawings scarcely suggest a resemblance to promycelia. In more than twenty figures Clinton (5) shows the various stages of germination which he studied. He also notes an occasional septum in some of the tubes, but he did not obtain the production of sporidia. Tranzschel (18) and Clinton (6) both claim to have established by infection experiments that *C. nitens* is the aecidial stage of *Puccinia Peckiana* Howe. Tranzschel's experiments, however, were not guarded by checks. The data given below show that such a connection is very improbable. The work of these authors has, nevertheless, been accepted and Arthur (1) has made *C. interstitiale* Schlecht. (*C. nitens* Burrill) the type species of his genus *Gymnoconia* Lagerh. This genus is described as autoecious and as including in its cycle of development, spermatia, aecidiospores and teleutospores. The only other species in the genus is *Gymnoconia Rosae-gymnocarpae* (Dietel.) Arthur. The teleutostage of this species is not known and my work suggests that a study of the germination of its aecidiospores may show it to be a short-cycled rust with a life history similar to that of *Caeoma nitens*. At any rate it is plain *C. nitens* cannot properly be included under Lagerheim's genus *Gymnoconia*.

Olive (16) and Kurssanow (11) have found that the aecidiospores of *Caeoma nitens* normally contain two nuclei. They have also shown that sexual fusions occur in the base of the caeoma, previous to the production of spores. The nuclear phenomena covering this stage in its life history are well established and agree with what has been found by Blackman (2), Christman (4) and others in a rather large number of rusts. In my preliminary paper I showed that the aecidiospores produce promycelia, but I had not then made a cytological study of the nuclear phenomena of their germination.

The material used in my further studies was obtained from well-infected blackberry leaves. The spores were dusted on the surface of tap-water in Petri dishes. In the early spring good germination could be obtained in such cultures when they were kept at room temperature (about 23° C.), but later in the season when the weather became quite warm it was necessary to place the cultures on top of an icebox in order to secure abundant germination. Most of the spores which I have used were taken from leaves of *Rubus frondosus* that were

gathered in Van Cortlandt Park, New York City. I have, however, also germinated spores that were sent to me from Urbana, Illinois; Columbia, Missouri, and Madison, Wisconsin. I find that they all produce promycelia.

Spores having promycelia in various stages of development were floated from tap-water cultures on to glass slides. They were attached to the slides by means of egg albumen, fixed, hardened in alcohol and stained with the triple stain. The ripe ungerminated aecidiospores seem difficult to fix and it was necessary to make many preparations in order to secure a sufficient number of slides that show the nuclei clearly defined and well stained. This difficulty in the fixation of the ungerminated spores is probably due to the slowness with which the fixative penetrates their dry cell walls. As soon as this wall is penetrated by the promycelial tube, the contents of the spore become easy to fix and readily stain.

In agreement with the observations of Olive (16) and Kurssanow (11), I find that the normal aecidiospores are binucleate. A study of stained sections through the caeoma, however, shows that, as Olive and Kurssanow have already observed, occasionally there are rows of spores, each of which contain more than two nuclei. Such spores are larger than the binucleate spores and their size is roughly proportional to the number of nuclei which they contain, thus maintaining nucleo-cytoplasmic ratio. Kurssanow found that these spores arise through a fusion of more than two cells in the caeoma. Two of these abnormal spores, one containing three nuclei and the other containing four nuclei, are shown in figures 1 and 2. A normal binucleate spore is shown in figure 3.

A study of the binucleate spores during germination shows that they become uninucleate previous to the production of promycelia. Preparations made from cultures that were fifteen hours old show a large number of uninucleate spores. In some of these preparations more than ninety per cent. of the spores contain only one nucleus, thus demonstrating that during the early stages of germination the binucleate spores become uninucleate. This observation strongly suggests that a nuclear fusion takes place. A large number of spores have been studied with the hope that the actual stages in such fusions could be demonstrated. The nuclei are clearly defined and can frequently be observed to lie close together and flattened against each other, but the actual breaking down of the membranes and the union of the

chromatin reticula are hard to demonstrate. I find no evidence of disintegrating nuclei in any of the spores.

The normal germination of the aecidiospore consists in the pushing out of a germ tube into which the protoplasmic contents of the spore passes. See figure 5. As the nucleus travels out into the germ tube it becomes elongated, figure 6. This nucleus soon divides, figure 7, producing two nuclei which may again divide immediately as shown in figure 8. The two nuclei that are the product of the first division may round up as shown in figure 9 and may even become separated by a cross wall before the second division occurs. Figure 10 shows a spore containing two nuclei and producing a germ tube. This suggests that here as in *Endophyllum sempervivi* according to Hoffmann the first nuclear division may occur in the spore.

Nuclear division, as noted, is sometimes followed immediately by cell division, figure 11, but in other cases the four nuclei of the promycelium may be present before cross walls are formed. There is some evidence that these cell divisions take place by constriction, as has been reported in other rusts (9 and 16), but I have not studied this point especially. The second nuclear division is soon followed by cell division giving normally four cells that are filled with protoplasm and contain one nucleus each, together with a stalk cell which is without a nucleus. The stalk cell is either empty or contains a very small amount of protoplasm at or near its upper end. We have here a case in which a cell is cut off which contains no nucleus and may or may not contain protoplasm. When protoplasm is present it is massed at the upper end of the cell leaving not even a vacuole and primordial utricle in the lower end. I have occasionally observed promycelia that are composed of more than five cells. Such promycelia, however, never produce more than four sporidia and when they are stained show a nucleus in only four of their cells. Brefeld (3) has observed a similar cutting off of functionless cells in the promycelia of *Tilletia Caries*, *Ustilago Maydis* and other forms.

The sterigmata arise as pointed protrusions from the nucleated cells of the promycelium. They are generally though not always produced near the distal end of the promycelial cell. After reaching a length of from fifteen to twenty microns the extreme end of the sterigma begins to enlarge. Thus the sporidium arises through the enlargement of the end of the sterigma. At first this enlargement is almost spherical, but it soon takes on the shape of the mature sporidium.

The nucleus enters the sporidium when it has reached approximately one half the size that it will be when mature. In passing through the sterigma the nucleus becomes very much elongated, as shown in figure 12. This elongated nucleus soon becomes spherical again and takes a position near the center of the sporidium. The mature sporidium contains all or almost all of the protoplasm from the promycelial cell which is left practically empty. Generally the sporidium falls from the sterigma before its nucleus begins to divide, but sometimes, as shown in figure 13, nuclear division occurs while it is still attached to the sterigma. Nuclear division may occur in the sporidium, either before or after germination. It is, however, generally binucleate at the time it germinates. It produces either a germ tube or a sterigma-like outgrowth on which is borne a secondary sporidium. Figure 14 shows a mature sporidium in which nuclear division has not yet occurred. The nucleolus is shown outside of the nucleus in this figure. This is probably the result of fixation, since the nuclei in many of the sporidia contain nucleoli. Figure 15 shows a sporidium which contains two nuclei.

It is worth noting that if the aecidiospores are immersed in water rather than floated on its surface, they produce long tubes which are often devoid of septa. Such promycelia seldom produce sporidia and might easily be mistaken for ordinary aecidiospore germ tubes. In cultures as much as two days old, these tubes regularly contain four nuclei. A study of earlier stages of germination shows that these four nuclei arise through two successive divisions of a single nucleus. This observation establishes the promycelial nature of these germ tubes also. They are therefore not to be confused with the germ tubes of ordinary aecidiospores. Werth (20) observed similar non-septate germ tubes when he immersed the aecidiospores of *Endophyllum sempervivi* in water and is inclined to the view that under these conditions the aecidiospores produce ordinary germ tubes rather than promycelia. My observations suggest that a cytological study of the aecidiospores of *E. sempervivi* which have been germinated under water will show that they produce only promycelia. Maire (12) claims to have found a variety of *E. sempervivi* (*E. sempervivi* variety *aecidioides* R. Maire) with aecidiospores that regularly produce germ tubes rather than promycelia, but it is possible that a further study may show that these germ tubes also are in reality promycelia.

In the light of the facts described, there can be no doubt that

Caecoma nitens is a short-cycled rust which has no connection with *Puccinia Peckiana*, but it has seemed desirable to make further infection experiments and to determine the nature of the mycelium that bears the teleutospores of *Puccinia Peckiana*. Clinton (5) figures the germination of these spores but he does not show the production of sporidia and from his drawings we cannot be sure that they are producing promycelia. I have, therefore, attempted to obtain further evidence along these lines.

On May 15, three blackberry plants that were badly infected with *Caecoma nitens* were removed from Van Cortlandt Park and placed in pots in a greenhouse. Nine uninfected plants were also brought in and after they had started new growth and appeared to be in a healthy condition, five of them were, on May 28, heavily inoculated with fresh mature aecidiospores of *Caecoma nitens*. They were kept under bell jars for a few days in order to secure a moist atmosphere for the germinating spores. The other four uninfected plants were used as checks. The five plants that were thus inoculated, as well as the three plants that were badly infected at the time they were placed in the greenhouse, are at present in a vigorous condition and show no signs of being infected with *Puccinia Peckiana*. I have also had under observation several hundred plants in Van Cortlandt Park that were badly infected with *Caecoma nitens* during the early spring. All of these plants were free from *Puccinia Peckiana* at the end of the growing season and I have not been able to find this rust in the vicinity of New York City.

To secure material of *Puccinia Peckiana* for study, a visit was made to a region near Old Forge, New York, in the Adirondack Mountains and to the vicinity of Glen, New Hampshire, in the White Mountains. In both of these regions, during the latter part of August, I found *Puccinia Peckiana* widely distributed and abundant on the leaves of *Rubus frondosus* Bigel. Stained sections of infected leaves, made from material obtained on this trip, show that the vegetative cells of the mycelium are binucleate, figure 16. The cells of the teleutospores in young sori contain two nuclei, but in the older sori, the cells have become uninucleate, presumably by the fusion of the two nuclei. Thus there is nothing unusual in the development of these teleutospores.

Some of the teleutospores were also germinated. They were scraped from green blackberry leaves and were spread over the surface

of agar media or floated on tap-water. After an incubation period of about two days, approximately thirty per cent. of the teleutospores germinated. They germinate by producing a promycelium that normally consists of five cells, one stalk cell and four cells that bear sporidia. Both cells of the teleutospore may produce promycelia simultaneously as shown in figure 17, but more often only one cell germinates, figure 18. The mature sporidium falls off from its sterigma and under favorable conditions germinates immediately. The sporidium produces either a germ tube or a sterigma on which is borne a secondary sporidium. A germinating sporidium is shown in figure 19. In some cases, a secondary sporidium may be borne on a branch of the germ tube, as is shown in figure 20. The development and germination of these spores leaves no doubt that they are true teleutospores, and that the aecidium stage of *Puccinia Peckiana* is yet to be determined.

While collecting in the regions referred to above, I found the blackberries quite commonly infected with a rust in the uredostage. These spores correspond fairly well with the description given by Fischer (7) for the uredospores of *Phragmidium violaceum* (Schultz) Winter, and there are in the herbarium of the New York Botanical Garden, specimens collected by Prof. L. M. Underwood at Glen, N. H., and labeled *P. violaceum*. Since, however, the teleutospores of this species has not been found in this country, the determination of the uredospores must be regarded as doubtful and there is a possibility that they belong with *Puccinia Peckiana*.

The life history of *Caeoma nitens* may be outlined as follows. The sporidia belong to the gametophytic generation and produce a mycelium that is composed of uninucleate cells. Whether the germ tubes of the sporidia are able to enter various tissues of the host or can only infect the young buds of the underground stem as is believed by Müller (13) to be the case for *Endophyllum Euphorbiae silvaticae*, is a question which must be left to future studies. The uninucleate mycelium is certainly perennial in the tissues of the host (14) and produces spermatogonia and aecidia on the shoots and leaves in the early spring. The aecidiospores are borne in chains and alternate with intercalary cells. Both the aecidiospores and intercalary cells are binucleate and constitute, therefore, a sporophytic generation. This binucleate condition arises through the fusion of equal cells in the base of the caeoma. With this fusion the sporophyte generation begins. In some cases

more than two cells fuse and this leads to the production of aecidiospores that contain more than two nuclei. The behavior of the nuclei in these spores during germination is a problem which I have not yet been able to solve. The age of the mycelium which bears the aecidiospores is not known. It is generally supposed to be approximately one year old but this question cannot be settled without experimental evidence. It is interesting to note in this connection that Müller found that the mycelium which produces most of the aecidiospores of *Endophyllum Euphorbiae silvaticae* is two years old. The binucleate aecidiospores become uninucleate, presumptively by nuclear fusions during the early stages of germination. Two successive divisions of the nucleus of the aecidiospore produce the four nuclei of the promycelium and each nucleated cell of this promycelium produces a sporidium.

I am inclined to believe that *Caeoma nitens* is a rather primitive form among the rusts. The predominance of the gametophytic generation over the sporophytic generation is strong evidence in favor of this view. This is the condition which we generally meet with in the lower forms of plant life. Those rusts in which the sporophytic generation has become the important stage are probably higher in the scale of evolution than such forms as *Caeoma nitens* and *Endophyllum sempervivi*. *Caeoma nitens* should also be regarded as more primitive than *Endophyllum*, since it possesses a simpler form of aecidium.

I am greatly indebted to Dr. R. A. Harper and Dr. W. G. Marquette for advice and criticism while engaged in this work.

COLUMBIA UNIVERSITY,
NEW YORK CITY

BIBLIOGRAPHY

1. Arthur, J. C. North American flora. Part 3, 7: 180-182. 1912.
2. Blackman, V. H. On the fertilization, alternation of generations, and general cytology of the Uredineae. Ann. Bot. 18: 323-373. 1904.
3. Brefeld, O. Botanische Untersuchungen über Schimmelpilze. Vol. 5, Plates 4 and 12. 1883.
4. Christman, A. H. Sexual reproduction in the rusts. Bot. Gaz. 29: 267-274. 1905.
5. Clinton, G. P. Orange rust of raspberry and blackberry. Ill. Agric. Exp. Sta. Bull. 29: 273-300. 1893.
6. Clinton, G. P. Relationship of *Caeoma nitens* and *Puccinia Peckiana*. Bot. Gaz. 20: 116. 1895.
7. Fischer, Ed. Die Uredineen der Schweiz. Vol. 2, pp. 416-418. 1904.

8. **Hoffmann, A. W. H.** Zur Entwicklungsgeschichte von *Endophyllum sempervivi*. Centralbl. f. Bak. 2 abt. **32**: 137-158. 1912.
9. **Holden, R. J., and Harper, R. A.** Nuclear divisions and nuclear fusion in *Coleosporium Sonchi-Arvensis* Lev. Trans. Wis. Acad. Sci. **14**: 63-77. 1903.
10. **Kunkel, O.** The production of a promycelium by the aecidiospores of *Caeoma nitens* Burrill. Bull. Tor. Bot. Club **40**: 361-366. 1913.
11. **Kurssanow, L.** Zur Sexualität der Rostpilze. Zeits. Bot. **2**: 81-93. 1910.
12. **Maire, R.** L'évolution nucléaire chez les *Endophyllum*. Jour. de Bot. **14**: 369-382. 1900.
13. **Müller, W.** Der Entwicklungsgang des *Endophyllum Euphorbiae silvaticae* (DC.) Winter und der Einfluss dieses Pilzes auf die Anatomie seiner Nahrungspflanze *Euph. Amygdaloides*. Centralbl. f. Bak. 2 Abt. **20**: 333. 1908.
14. **Newcombe, F. C., and Galloway, B. T.** Perennial mycelium of the fungus of the blackberry rust. Jour. Mycol. **6**: 106-107. 1890.
15. **Nypels, P.** La germination de quelque écidiospores. Ann. Soc. Belge de Micros. **22**: 101-111. 1898.
16. **Olive, E. W.** Sexual cell fusions and vegetative nuclear divisions in the rusts. Ann. Bot. **22**: 331-360. 1908.
17. **Sappin-Trouffy, P.** Recherches histologiques sur la famille des *Uredinées*. Le Botaniste **5**: 59-244. 1896.
18. **Tranzschel, W.** Culturversuche mit *Caeoma interstitiale* Schlecht. (= *C. nitens* Schw.). *Hedwigia* **32**: 257-259. 1893.
19. **Tulasne, L. R.** Second memoire sur les *Uredinees* et les *Ustilaginees*. Ann. Sci. Nat. IV. **2**: 77. 1854.
20. **Werth, E.** Zur Kenntniss des *Sempervivum-Rostes*. Centralbl. f. Bak. 2 Abt. **36**: 395-408. 1913.

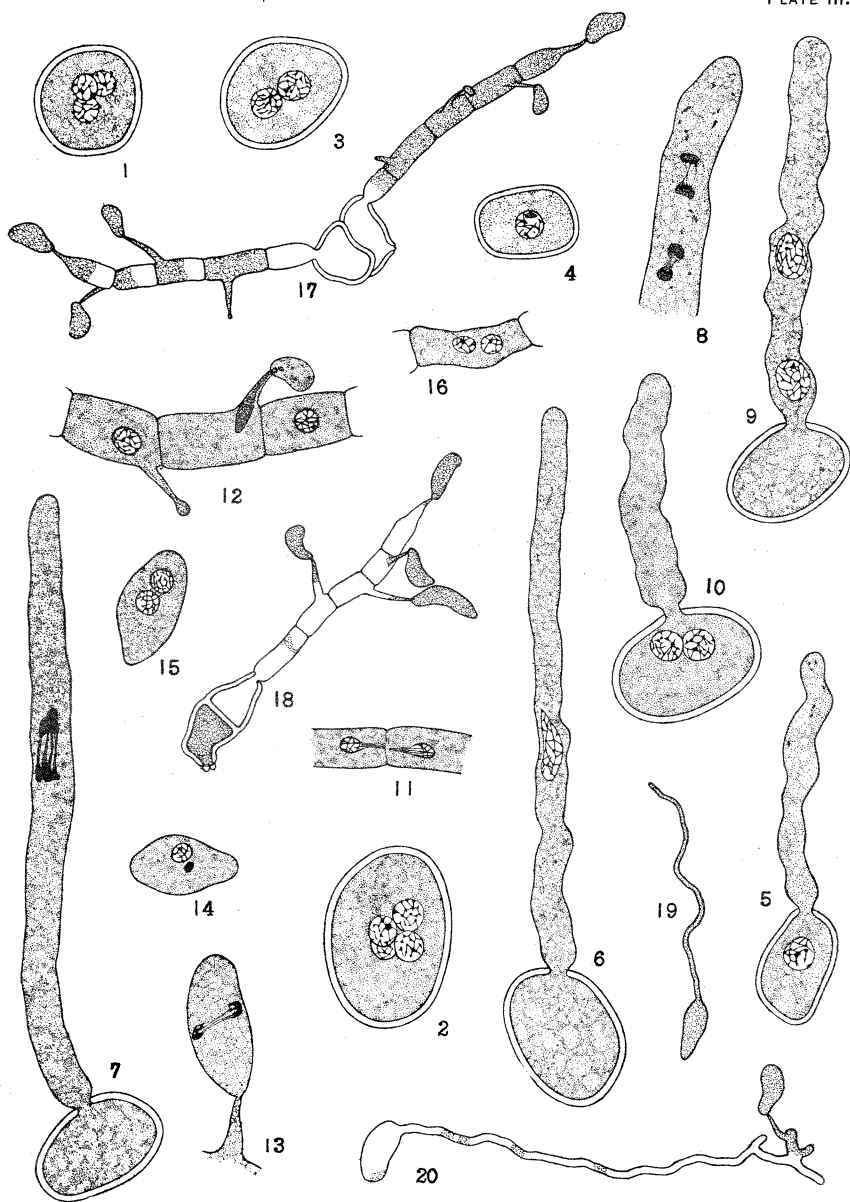
EXPLANATION OF PLATE III

All the figures were drawn with the aid of a camera lucida, and with the Leitz Obj. $\frac{1}{8}$ inch. or $\frac{1}{12}$ inch, and ocular 4.

Caeoma nitens

Magnification 1570 diameters

- FIG. 1. An aecidiospore which contains three nuclei.
 FIG. 2. An aecidiospore which contains four nuclei.
 FIG. 3. A normal aecidiospore containing two nuclei.
 FIG. 4. An aecidiospore which has become uninucleate.
 FIG. 5. A uninucleate aecidiospore in an early stage of germination.
 FIG. 6. A later stage of germination in which the nucleus is much elongated and is passing up the promycelial tube.
 FIG. 7. First nuclear division, diaster.
 FIG. 8. The second nuclear division in telophase.
 FIG. 9. A promycelial tube containing two nuclei that have rounded up after the first division.
 FIG. 10. An aecidiospore containing two nuclei and producing a promycelial tube. This figure suggests that the first division has taken place in the spore.



KUNKEL: NUCLEAR BEHAVIOR IN PROMYCELIA.

FIG. 11. Cell division following immediately after the second nuclear division.

FIG. 12. Passage of the elongated nucleus into the sporidium.

FIG. 13. Nuclear division taking place in a sporidium that is still attached to its sterigma.

FIG. 14. A uninucleate sporidium, showing the nucleolus outside the nucleus. This is probably a result of fixation.

FIG. 15. A binucleate sporidium, showing the two nuclei rounded up and in a resting condition.

Puccinia Peckiana

Magnification 1000 diameters

FIG. 16. A typical vegetative cell from the mycelium, showing the binucleate condition.

FIG. 17. A germinating teleutospore. Both cells of this teleutospore are producing promycelia simultaneously.

FIG. 18. A teleutospore which shows only one of its cells germinating.

FIG. 19. A sporidium which has produced a germ tube.

FIG. 20. A sporidium which has produced a germ tube on which is borne a secondary sporidium.